Patent Claims

1. A method for the detection of cytosine methylation in DNA samples, characterized in that the following steps are conducted:
a genomic DNA sample which comprises target DNA and background DNA is chemically treated such that all unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged;
the chemically treated DNA sample is amplified with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, the composition of which leads to a preferred amplification of the target DNA over the background DNA; and the methylation state in the target DNA is concluded from the presence of an amplificate or its quantity.

2. The method according to claim 1, further characterized in that the nucleotide mixture only contains 2'-deoxyguanosine triphosphate (dGTP), 2'-deoxyadenosine triphosphate (dATP) and 2'-deoxythymidine triphosphate (dTTP).

3. The method according to claim 2, further characterized in that the nucleotide mixture additionally contains a comparatively small concentration of 2'-deoxycytidine triphosphate (dCTP).

4. The method according to claim 3, further characterized in that the initial concentration of dCTP for the amplification is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture.

5. The method according to claim 1, further characterized in that the nucleotide mixture contains only 2'-deoxycytidine triphosphate (dCTP), 2'-deoxyadenosine triphosphate (dATP) and 2'-deoxythymidine triphosphate (dTTP).

6. The method according to claim 5, further characterized in that the nucleotide mixture additionally contains a comparatively small concentration of 2'-deoxyguanosine triphosphate (dGTP).

7. The method according to claim 6, further characterized in that the initial concentration of dGTP for the amplification is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture.

8. The method according to one of claims 2 to 7, further characterized in that 2'-deoxyuridine triphosphate is utilized instead of 2'-deoxythymidine triphosphate.

9. The method according to one of the preceding claims, further characterized in that terminating dideoxynucleotides are additionally used in the amplification.

10. The method according to one of the preceding claims, further characterized in that the denaturing temperature lies below 90 °C in the PCR amplification.

11. The method according to one of the preceding claims, further characterized in that the sample DNA is obtained from serum, plasma, urine, sputum or other body fluids of an individual.

12. The method according to one of the preceding claims, further characterized in that the chemical treatment is conducted with a bisulfite, disulfite or hydrogen sulfite containing solution.

13. The method according to claim 12, further characterized in that the chemical treatment is conducted after embedding the DNA in agarose.

14. The method according to claim 12, further characterized in that in the

chemical treatment, a reagent that denatures the DNA duplex and/or a radical scavenger is present.

15. The method according to one of the preceding claims, further characterized in that the amplification is conducted in the presence of at least one other oligonucleotide or a PNA oligomer, which binds to a 5'-CG-3' dinucleotide or a 5'-tG-3'-dinucleotide or a 5'-Ca-3' dinucleotide, whereby the other oligonucleotide or PNA oligomer preferably binds to the background DNA and adversely affects its amplification and wherein "t" represents a thymine at a position which correlates with an unmethylated cytosine prior to bisulfite treatment and "a" correlates with such a thymine position.

16. The method according to claim 15, further characterized in that this binding site of the other oligonucleotide or PNA oligomer overlaps with the binding sites of the primers on the background DNA, and said other oligonucleotide or PNA oligomer thus impedes the binding of at least one primer oligonucleotide to the background DNA.

17. The method according to one of claims 15 or 16, further characterized in that at least two other oligonucleotides or PNA oligomers are used, whereby their binding sites again each overlap with the binding site of a primer to the background DNA and said other oligonucleotides and/or PNA oligomers thus impede the binding of both primer oligonucleotides to the background DNA.

18. The method according to one of claims 15 to 17, further characterized in that these other oligonucleotides and/or PNA oligomers are present in at least five times the concentration of the primer oligonuleotides.

19. The method according to one of the preceding claims, further characterized in that the polymerase used has no 5'-3' exonuclease activity.

20. The method according to one of the preceding claims, further characterized in that the other oligonucleotides are modified at the 5'-end and thus cannot be significantly degraded by a polymerase with 5'-3' exonuclease activity.

21. The method according to one of the preceding claims, further characterized in that the primers in the amplification distinguish between target DNA and background DNA.

22. The method according to claim 21, further characterized in that the background DNA is methylated, while the target DNA is unmethylated, each at positions at which at least one primer for the amplification binds, whereby the one or more primers preferably bind to the target DNA after the chemical treatment.

23. The method according to one of the preceding claims, further characterized in that additionally at least one reporter oligonucleotide is used in the amplification whose fluorescence properties change as a consequence of the amplification.

24. The method according to claim 21, further characterized in that a Taqman assay or a LightCycler assay or an assay with the use of Molecular Beacons is conducted to conclude upon the methylation state at the last step of the method.

25. The method according to one of claims 23 or 24, further characterized in that the reporter oligonucleotide bears at least one fluorescent label.

26. The method according to one of claims 18 to 22, further characterized in that the reporter oligonucleotide or the reporter oligonucleotides indicates or indicate the amplification either by an increase or a decrease in fluorescence.

27. The method according to claim 26, further characterized in that the increase or decrease in fluorescence is used directly for the analysis and a conclusion on the methylation state of the DNA to be analyzed is made from the fluorescent

signal.

28. The method according to one of the preceding claims, further characterized in that the background DNA is present in 100X the concentration in comparison to the target DNA.

29. The method according to one of the preceding claims, further characterized in that the background DNA is present in 1000X the concentration in comparison to the target DNA.

30. The method according to one of the preceding claims, further characterized in that a conclusion is made on the presence of a disease or another medical condition of the patient from the methylation degree of the different CpG positions investigated.

31. The method according to one of the preceding claims, further characterized in that the amplificates themselves bear a detectable label for the detection.

32. The method according to claim 31, further characterized in that the labels are fluorescent labels.

33. The method according to claim 31, further characterized in that the labels are radionuclides.

34. The method according to claim 31, further characterized in that the labels are removable mass labels which are detected in a mass spectrometer.

35. The method according to one of the preceding claims, further characterized in that during amplification, one of the primers is bound to a solid phase.

36. The method according to one of claims 1 to 34, further characterized in that

all the amplificates are detected in the mass spectrometer and are thus clearly characterized by their mass.

37. Use of a method according to one of the preceding claims for the diagnosis and/or prognosis of adverse events for patients or individuals, whereby these adverse events belong to at least one of the following categories: undesired drug interactions; cancer diseases; CNS malfunctions, damage or disease; symptoms of aggression or behavioral disturbances; clinical, psychological and social consequences of brain damage; psychotic disturbances and personality disorders; dementia and/or associated syndromes; cardiovascular disease, malfunction and damage; malfunction, damage or disease of the gastrointestinal tract; malfunction, damage or disease of the respiratory system; lesion, inflammation, infection, immunity and/or convalescence; malfunction, damage or disease of the body as [a consequence of] an abnormality in the development process; malfunction, damage or disorder of the skin, the muscles, the connective tissue or the bones; endocrine and metabolic malfunction, damage or disease; headaches or sexual malfunction.

38. Use of a method according to one of the preceding claims for the classification of patients into subgroups.

39. Use of a method according to one of the preceding claims for the differentiation of cell types or tissues or for the investigation of cell differentiation.

40. A kit consisting of a reagent containing a bisulfite, primers for the amplification and a nucleotide mixture according to one of claims 2 to 8.